*Mini-Review*

# Dysfunctional Nav1.5 channels due to SCN5A mutations

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**Impact statement**

The field of ion channelopathy caused by dysfunctional Nav1.5 due to SCN5A mutations is rapidly evolving as novel technologies of electrophysiology are introduced and our understanding of the mechanisms of various arrhythmias develops. In this review, we focus on the dysfunctional Nav1.5 related to arrhythmias and the underlying mechanisms. We update SCN5A mutations in a precise way since 2013 and presents novel classifications of SCN5A mutations responsible for the dysfunction of the peak ($I_{Na-P}$) and late ($I_{Na-L}$) sodium channels based on their phenotypes, including loss-, gain-, and coexistence of gain- and loss-of function mutations in $I_{Na-P}$, $I_{Na-L}$, respectively. We hope this review will provide a new comprehensive way to better understand the electrophysiological mechanisms underlying arrhythmias from cell to bedside, promoting the management of various arrhythmias in practice.

## Abstract

The voltage-gated sodium channel 1.5 (Nav1.5), encoded by the SCN5A gene, is responsible for the rising phase of the action potential of cardiomyocytes. The sodium current mediated by Nav1.5 consists of peak and late components ($I_{Na-P}$ and $I_{Na-L}$). Mutant Nav1.5 causes alterations in the peak and late sodium current and is associated with an increasingly wide range of congenital arrhythmias. More than 400 mutations have been identified in the SCN5A gene. Although the mechanisms of SCN5A mutations leading to a variety of arrhythmias can be classified according to the alteration of $I_{Na-P}$ and $I_{Na-L}$ as gain-of-function, loss-of-function and both, few researchers have summarized the mechanisms in this way before. In this review article, we aim to review the mechanisms underlying dysfunctional Nav1.5 due to SCN5A mutations and to provide some new insights into further approaches in the treatment of arrhythmias.

**Keywords:** Nav1.5, SCN5A, gain-of-function, loss-of-function, INa-P, INa-L

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## Introduction

The α-subunit (Nav1.5) encoded by the SCN5A gene is the predominant element in heart tissue and plays a critical role in the excitability of cardiomyocytes. Nav1.5 channels mediate the inward sodium current ($I_{Na}$) and induce fast depolarization, thereby initiating the excitation–contraction coupling cascades in the cells. $I_{Na}$ mediated by Nav1.5 can be classified into peak and late sodium currents ($I_{Na-P}$ and $I_{Na-L}$). Mutations of SCN5A can impair Nav1.5 function and change the magnitude and duration of $I_{Na-P}$ and $I_{Na-L}$, consequently leading to different types of fatal arrhythmias. Gain- or loss-of-function mutations are responsible for most of the pathogenesis of SCN5A mutation-induced cardiac disorders. More than 400 mutations have been identified in the SCN5A gene (updated SCN5A mutations from 2013 to 2018 are depicted in Figure 1). In this review, we will firstly introduce the biology of the Nav1.5 channel and then focus on the mechanisms underlying gain or loss of function and summarize arrhythmic consequences of mutant Nav1.5 and their clinical implications.

## Biology of the Nav1.5 channel

Sodium channels are hetero-multimeric proteins composed of a pore-forming α subunit and auxiliary β subunits. The α subunit consists of four homologous domains (DI–DIV). Each domain contains six transmembrane-spanning segments (S1–S6), of which the S4 segment functions as a voltage sensor and the S5 and S6 regions form the pore with the

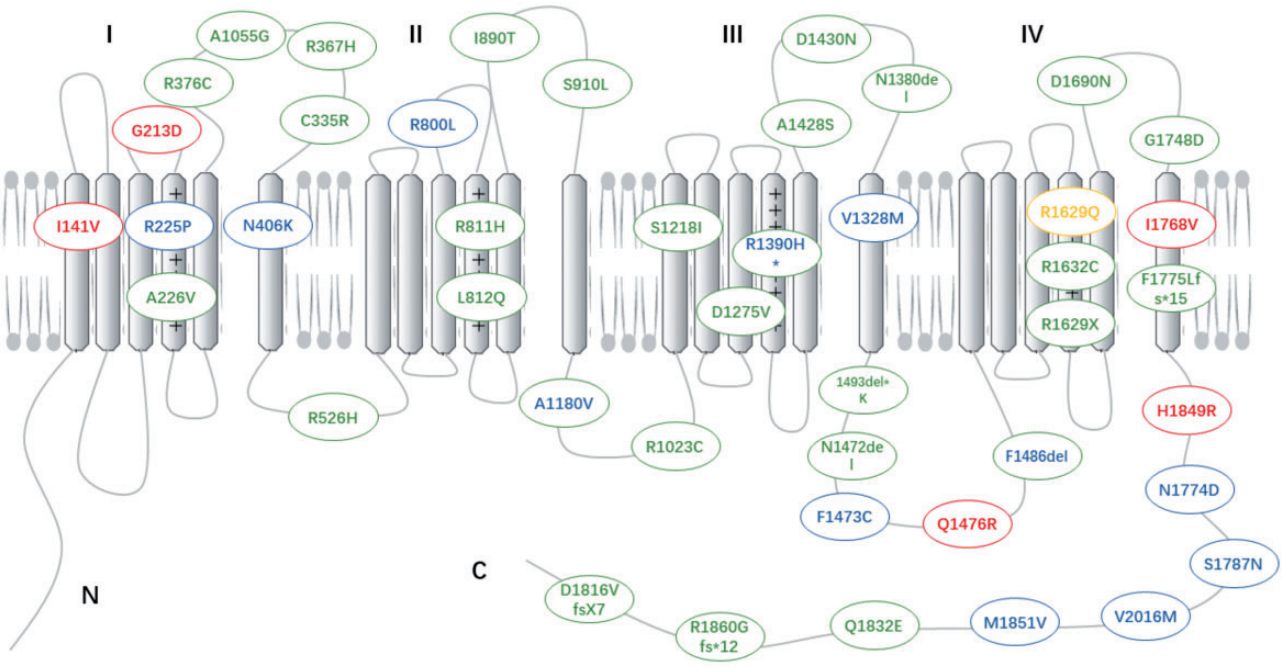


**Figure 1.** Updated SCN5A mutations identified from 2013 to present. The red represents gain of $I_{Na\text{-}P}$, blue represents gain of $I_{Na\text{-}L}$, green represents loss of $I_{Na\text{-}P}$, yellow represents loss of $I_{Na\text{-}L}$, the mixed colors represent coexistence of gain- and loss-of-function mutations. (A color version of this figure is available in the online journal.)

intermembrane P-loop.[1] The α-subunit (Nav1.5) encoded by the SCN5A gene is the predominant element in the heart and plays a critical role in the excitability of cardiomyocytes. In terms of its biophysical properties, Nav1.5 channels can be observed at three states: closed at resting membrane potential (approximately −85 mV), activated during depolarization, and inactivated. Transition between these states depends primarily on the transmembrane potential, time, temperature, and pH value. Recovery from inactivation occurs within the repolarization phase during diastole under physiological conditions. The upstroke speed of the AP and conduction is determined by the numbers of Nav1.5 channels that are available for opening. The inactivation process is rapid and stable for most ion channels. However, sodium channels may inactivate incompletely, therefore generating a so-called $I_{Na\text{-}L}$ throughout the plateau phase of the AP.[2] In addition, some channels may reactivate during the repolarizing phase of the AP at a range of potentials in which inactivation is not complete and exhibits overlap with activation, resulting in the "window current."[3] Both $I_{Na\text{-}L}$ and the window current can play critical roles in genetic and acquired cardiac diseases, as discussed below.

The SCN5A gene, which is expressed in a circadian pattern, is also expressed in extracardiac cells such as the excitable cells of the cerebral limbic system, and diverse subtypes of non-excitable cells, including microglia, astrocytes, T-lymphocytes, macrophages, fibroblasts, endothelial cells, and different type of cancer cells. Mounting evidence has demonstrated that sodium channels can take part in various effector functions and lead to non-classical effects in non-excitable cells. For instance, in cancer cells, Nav1.5 is related to enhanced invasiveness and metastasis. Nav1.5 affects $Na^+/H^+$ exchanger activity in breast cancer cells and causes local extracellular acidification, which results in activated cathepsin and consequently leads to the breakdown of the extracellular matrix. $Na^+$ inflow is equally important in this process, as blocking Nav1.5 channels decreases the invasion of cancer cells. In addition, Nav1.5 in endosomes of macrophages from individuals with multiple sclerosis contributes to phagocytosis and pH regulation. It is suggested that targeting Nav1.5 could be a putative therapeutic approach in this disease.[4]

The Nav1.5 channel mediates the rapid entry of the sodium current ($I_{Na}$), a current that mainly contributes to the depolarization of the action potential (AP) in cardiac myocytes and the His-Purkinje system.[5] $I_{Na}$ mediated by Nav1.5 can be classified into peak and late sodium currents ($I_{Na\text{-}P}$ and $I_{Na\text{-}L}$). $I_{Na\text{-}P}$ occurs during phase 0 of the AP with a density of approximately 391 uA/uF and is quickly inactivated within 1–2 ms. The $I_{Na\text{-}L}$ amplitude is much smaller than the $I_{Na\text{-}P}$ amplitude in many species (approximately 0.1%–1%) and is inactivated more slowly during the plateau of the AP[6] with the time constant ranging from 75 to 450 ms.[7,8] The $I_{Na\text{-}P}$ is mainly associated with the initiation of cardiac excitability and electrical conduction. The $I_{Na\text{-}P}$ drives the rapid AP upstroke, resulting in further channel activation. This transient increase in intracellular sodium leads to calcium current (ICa) influx via L-type voltage-gated channels when the voltage upstroke reaches approximately −25 mV. The depolarization-activated ICa induces $Ca^{2+}$ release from intracellular sarcoplasmic reticular $Ca^{2+}$ stores and initiates myocardial mechanical activity.[9]

## Pathogenesis of SCN5A mutations

SCN5A gene mutations impair Nav1.5 function and consequently change the magnitude and duration of $I_{Na\text{-}P}$ and $I_{Na\text{-}L}$, which lead to different types of fatal arrhythmias.[10] SCN5A mutations are responsible for various types of cardiac disorders, including Brudaga syndrome (BrS),[11] long QT syndrome 3 (LQT3),[12] cardiac conduction disease (CCD),[13] sick sinus syndrome (SSS),[14] atrial fibrillation (AF),[15,16] progressive cardiac conduction defect (PCCD), dilated cardiomyopathy (DCM),[17] multifocal ectopic Purkinje-related premature contraction (MEPPC),[18] and the onset of a variety of non-cardiac diseases, including bowel syndrome,[19] myotonic dystrophy,[20] epilepsy,[21] pain,[22] and ataxia.[23]

SCN5A mutations result in the dysfunction of Nav1.5 due to defective protein trafficking, targeting, fixation to specific cellular compartments, post-translational protein processing, the modulation of biophysical properties and many unclear mechanisms.[24] Genotype and phenotype vary significantly, as the phenotypic characterization ranges from asymptomatic phenotypes to sudden cardiac death (SCD) in individuals that carry the same mutations. In addition, specific SCN5A mutations cause an individual phenotype or compound phenotypes, indicating that a complex pathogenesis underlies SCN5A mutations.

## Gain-of-function mutations and arrhythmias

Long QT syndrome (LQTS) is characterized by prolonged ventricular repolarization, which predisposes individuals to develop torsades de Pointes (TdP) and SCD. LQTS3 is caused by gain-of-function mutations of SCN5A. Approximately 8–10% of patients with SCN5A mutations are positively phenotypic as having LQTS.[25,26] The first SCN5A mutation related to LQT3, the deletion of amino acids 1505–1507 (ΔKPQ), was identified by Wang *et al.*[27] According to previous reports, cardiac events primarily occurred during sleep in LQT3 patients, and 18% died suddenly.[28] The gain-of-function SCN5A mutation leads to enhanced $I_{Na\text{-}P}$ and $I_{Na\text{-}L}$, which finally triggers life-threating arrhythmias primarily in LQT3 patients.

## Gain-of-function mutations of $I_{Na\text{-}P}$

The underlying mechanisms of SCN5A mutations that lead to the gain-of-function of $I_{Na\text{-}P}$ are mainly due to abnormalities in mutation-induced kinetic properties, including augmented $I_{Na\text{-}P}$ amplitudes, negative shifts in the voltage-dependence of activation, and an increased speed of recovery from inactivation. The most recently identified SCN5A mutations over the last five years (from 2013 to 2018) are shown in Table 1.[29–34]

First, gains of channel function can be caused by variants that lead to augmented $I_{Na\text{-}P}$ amplitudes. LQT3 mutations, such as I1748V[31] and G1748D,[29] exhibited greater $I_{Na\text{-}P}$ than wild type, whereas variants A572D[35] and G615E[36] also showed a significant gain of function of $I_{Na\text{-}P}$, but with an unclear clinical phenotype. However, some SCN5A mutations identified in clinical LQTS patients, such as F1250L[37] and N406K[38] variants, showed no significant changes in $I_{Na\text{-}P}$ amplitudes. These phenomena suggested that although altered $I_{Na\text{-}P}$ amplitudes affect phenotypes of the diseases directly, there may be other unknown mechanisms that are related to certain environmental factors or unknown gene mutations that contribute to genotype–phenotype interactions.

Second, the gain of function of $I_{Na\text{-}P}$ could be generated by a negative shift in voltage-dependent activation potentials. It was reported that Nav1.5 reached its maximal current at −20 mV, while the variants G1748D,[29] H1849R,[30] S216L,[39] G983D, and F816Y[40] showed peak inward currents at a more negative voltage. Patients with G1748D and H1849R showed typical LQT3 features. However, patients that carried the variants S216L, G983D, and F816Y showed an unclear phenotype. These cases indicate that not all negative shifts of activation result in a gain of function of SCN5A and manifest the LQT3 phenotype; there must be other unidentified mechanisms that underlie genotype–phenotype interactions.

Third, gain of function of $I_{Na\text{-}P}$ can be induced by a faster recovery from inactivation. According to this underlying mechanism, variants A572D and G983D showed a faster resumption of inactivation due to a fast component of the recovery. Moreover, the dedication of the fast component to

**Table 1.** The newest identified gain of INa-P function of SCN5A mutations from 2013 to 2018 and their reported electrophysiological properties.

| Mutation | Protein domain | Biophysical properties of mutant protein | Cardiomyopathy and accompanied features | References |
|---|---|---|---|---|
| I141V | DI/S1 | Increased Iwindow: negative shift of activation | PVC, tachycardia | 33 |
| G213D | DI/S3-S4 | Increased INaP: negative shift of act, positive shift of inactivation | AA, VA, DCM | 34 |
| Q1476R | DIII-DIV | Increased INaP: positive shift of inactivation; increased INaL | LQT3 | 32 |
| G1748D | N-terminus DIV S6 | Increased INaP: positive shift of inactivation, accelerated recovery from inactivation | LQT3 | 29 |
| I1768V | DIV/S6 | Increased INaP, Iwindow: negative shift of the activation, faster recovery from inactivation | LQT3, SCD | 31 |
| H1849R | C-terminus | Increased INaP and INaL: negative shift of inactivation, slower inactivation | LQT, AF, VT, SCD | 30 |

AA: atrial arrhythmia; AF: atrial fibrillation; DCM: dilated cardiomyopathy; LQT: long QT syndrome; PVC: polymorphic ventricular complexes; SCD: sudden cardiac death; VA: ventricular arrhythmia; VT: ventricular tachycardia.

the recovery from inactivation was relatively augmented in G983D. The A572D variant manifested as atria tachycardia, and G983D manifested as an abnormal T-wave on the electrocardiography (ECG).[41–43] The configuration of the T-wave pointed out the differences in the time course of ventricular repolarization. Morphologic changes in the T wave are sometimes more immediately remarkable than the mere prolongation of the QT interval; in some cases, the morphology of the T-wave is the only sensitive sign of ventricular repolarization disturbances.[44,45]

## Gain-of-function mutations of $I_{Na\text{-}L}$

$I_{Na\text{-}L}$ has also been called steady-state $I_{Na}$, slow inactivation, persistent current, and late current. Under physiological conditions, the amplitude of $I_{Na\text{-}L}$ is larger in mid-myocardial cells (M cells) and Purkinje fibers than epicardial and endocardial cells.[46] Although the magnitude of persistent $I_{Na\text{-}L}$ is negligible compared to $I_{Na\text{-}P}$ (0.1%–1%), the delay in inactivation breaks the delicate equilibrium of inward and outward currents, resulting in a prolongation of the action potential duration (APD), which manifests as a prolonged QT interval on ECG.[7]

An increase in $I_{Na\text{-}L}$ due to acquired conditions or inherited SCN5A mutations in favor of intracellular $Ca^{2+}$ loading,[47,48] the occurrence of early and delayed after depolarization (EAD and DAD),[49,50] triggered activities,[51] and spontaneous diastolic depolarization[52] that promotes the spatial and temporal dispersion of ventricular repolarization can lead to reentrant arrhythmias (Figure 2).

The detrimental effects of a pathological persistent $I_{Na\text{-}L}$ contribute to the development of arrhythmic disorders. The mechanisms are as follows:

(i) During phase 2 of the AP plateau, membrane resistance is high while the ionic conductance is low,[53] which caused marked APD prolongation. A prolonged APD helps L-type $Ca^{2+}$ channels recover from inactivation and reactivate to form the upstroke of an EAD during the AP plateau.[54] (ii) An increase in $I_{Na\text{-}L}$ due to delayed inactivation increased intracellular $Ca^{2+}$ entry via the $Na^+/Ca^{2+}$ exchanger (NCX, 3 $Na^+$ out, 1 $Ca^{2+}$ in)[55,56] and interacted with calmodulin in a protein kinase II (CaMKII)-dependent manner,[51,57] which had a positive feedback on Na+ loading. Additionally, it increased sarcoplasmic reticulum (SR) $Ca^{2+}$ loading-induced $Ca^{2+}$ release.[47,58] The increases in $I_{Na\text{-}L}$, the activity of CaMKII and SR $Ca^{2+}$ release contributed to a substrate precipitating DAD.[59] (iii) Diastolic depolarization during phase 4 of the AP usually occurred in spontaneous pace-making cells of the sinoatrial and atrioventricular nodes.[60] However, spontaneous diastolic depolarizations were often observed in Purkinje fibers and atrial tissue isolated from a diseased heart with a persistent $I_{Na\text{-}L}$. According to reports, the gain of function of SCN5A may lead to spontaneous AP firing and abnormal automaticity, especially in myocytes that were relatively depolarized and had low resting $K^+$ conductance.[61] After all, the formation of EAD and DAD due to the gain of function of $I_{Na\text{-}L}$ occurred more frequently in M cells than endo and epi and increased the transmural dispersion of ventricular repolarization, which finally caused reentrant arrhythmic events, which manifested as TdP and ventricular fibrillation (VF).

Previous studies revealed several gain-of-function mechanisms of $I_{Na\text{-}L}$ with an alteration in channel kinetics, including a slower speed of inactivation or a positive shift in the voltage-dependence of inactivation (Table 2).[62–70]

First, gain-of-function mutations of SCN5A resulted in slower inactivation kinetics and increased $I_{Na\text{-}L}$, which included the LQT3-causing variant A993T as well as A572D[43] and K480N[42] in patients with an unclear

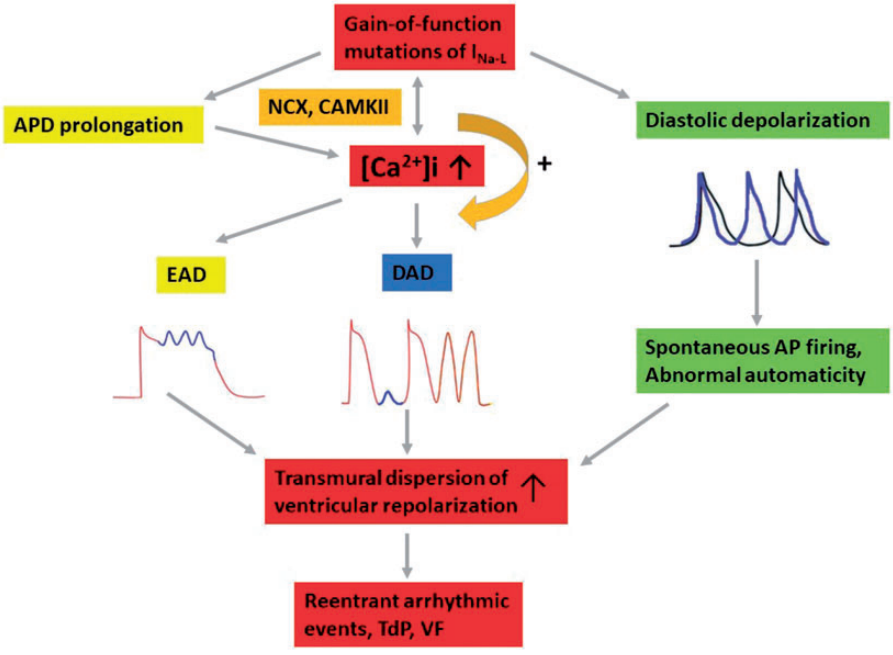


**Figure 2.** The ion mechanism of gain of function of $I_{Na\text{-}L}$ leading to arrhythmia.

**Table 2.** The newest identified gain of INa-L function of SCN5A mutations from 2013 to 2018 and their reported electrophysiological properties.

| Mutation | Protein domain | Biophysical properties of mutant protein | Cardiomyopathy and accompanied features | References |
|---|---|---|---|---|
| R225P | DI/S4 | Increased INaL and Iwindow: slower inactivation, shallower activation curve slope | Multifocal Ventricular Ectopy-associated Cardiomyopathy, LQT | 66 |
| N406K[a] | DI/S6 | Decreased INaP: positive shift of activation; increased INaL | BrS, LQT | 65 |
| R800L | DII/S3-S4 | Increased INaL, Iwindow: incomplete inactivation and slowed decay of currents | LQTS, in compound with A261V-SNTA1 | 64 |
| A1180V[a] | DII-DIII | Decreased INaP: negative shift of inactivation; increased INaL | DCM, AVB | 63 |
| V1328M | DIV/S6 | Increased INaL: positive shift of inactivation | drug-induced BrS | 69 |
| F1473C | DIII-DIV | Increased INaL, Iwindow: negative shift of the inactivation | LQT3, TdP, VT, AVB | 62 |
| F1486del[a] | DIII-DIV | Decreased INaP; increased INaL: positive shift of inactivation, negative shift of activation, slower Na+ current decay | BrS, LQT | 65 |
| N1774D | C-terminus | Increased INaL: negative shift of activation, slower Na+ current decay; increased INaP | LQT | 65 |
| S1787N | C-terminus | Increased INaL due to splice variant and environmental factors | LQT3 | 67 |
| M1851V | C-terminus | Increased INaL: slower inactivation, faster recovery from inactivation, positive shift of inactivation | AF, VA | 70 |
| V2016M[a] | C-terminus | Increased INaL: PKA activation; decreased INa | LQT, SND | 68 |

[a]Both gain and loss of function mutations;
AF: atrial fibrillation; AVB: atrioventricular block; BrS: Brugada syndrome; DCM: dilated cardiomyopathy; LQT: long QT syndrome; SND: sudden nocturnal death; TdP: Torsade de pointes; VA: ventricular arrhythmia; VT: ventricular tachycardia

phenotype. In a patient with a mutation of T1526P,[71] the cardiac examination was nearly normal, but T1526P showed a fractional gain-of-function property by a reduced speed of inactivation.

Second, gain of function can be caused by a positive shift in the voltage-dependence of inactivation, which also increased the magnitude and duration of $I_{Na\text{-}L}$. For example, the LQT3 mutation F1486L,[72] which results in a gain of function, showed a positive shift in the voltage-dependence of inactivation. This effect of the N1325S[73,74] variant with an unclear phenotype was also observed in patients.

## Loss-of-function mutations and arrhythmias

SCN5A loss-of-function mutations often cause BrS which is characterized by ST-segment elevation in the right precordial leads (V1–V3). Over 300 SCN5A loss-of-function mutations have been identified in connection with BrS.[75,76] Misfolded channels, trafficking defects, and negatively shifted steady-state inactivation curves contribute to a reduced availability of functional Nav1.5 channels on the plasma membrane.[77]

CCD mutations were also related to loss of function in Nav1.5.[78] SCN5A loss of function reduced the AP upstroke velocity, which further delayed the rapid conduction of the electrical impulse through the highly specialized conduction system.[79] Most SCN5A loss-of-function mutations prolonged the rising time of the AP and rendered it more difficult to reach the membrane potential, which is necessary for the fast AP upstroke. In addition, defects in channel gating kinetics, an inability to conduct sodium, and channel retention in the ER would further reduce the availability of channels. The SCN5A mutations that lead to a loss of channel functions can be classified as follows:

## Loss-of-function mutations of $I_{Na\text{-}P}$

Mutations that lead to a loss of function of $I_{Na\text{-}P}$ are related to an alteration in channel kinetics, including decreased $I_{Na\text{-}P}$ amplitudes and retention in the ER, but the function of the channels was restored when they reached the membrane (Table 3).[80–107]

Decreased current amplitudes cause loss of function of Nav1.5. As shown in the variants R222stop and R2012H,[42] patients diagnosed with BrS matched the loss of function with a reduction of $I_{Na\text{-}P}$. In addition, there were a number of SCN5A mutations, such as E161K[108] and P336L,[109] that exhibited a dramatic reduction in $I_{Na\text{-}P}$ density, while the kinetics and gating properties of the mutant channels were unaffected. These findings demonstrated that the decrease in $I_{Na\text{-}P}$ density of the mutant channels was primarily caused by their retention in the ER, but the function of the channels was restored when they reached the membrane. Thus, we hypothesized that amino acid mutations might affect the protein structure, which would lead to misfolding and ER retention.

## Loss-of-function mutations of $I_{Na\text{-}L}$

Loss of SCN5A channel function manifesting as ECG ST-segment elevation in right precordial leads was thought to contribute to an early repolarization of the right ventricular sub-epicardial myocardium that differentially altered

**Table 3.** The newest identified loss of INa-P function of SCN5A mutations from 2013 to 2018 and their reported electrophysiological properties.

| Mutation | Protein domain | Biophysical properties of mutant protein | Cardiomyopathy and accompanied features | References |
|---|---|---|---|---|
| A226V | DI/S4 | Decreased INaP | BrS, in compound with p; R1629X | 102 |
| C335R | DI/S5-S6 | Decreased INaP | AF, BrS | 107 |
| D349N | | No detected current | SSS | 89 |
| R367G | | Decreased INaP: trafficking defect | CCD | 40 |
| R367H | DI/S5-S6 | Decreased INaP: positive shift of activation, negative shift of inactivation, faster recovery from inactivation | BrS, SUNDS | 106 |
| R376C | DI/S5-S6 | Decreased INaP: positive shift of activation | SSS, SCD | 91 |
| R526H | DI/DII | Decreased INaP: trafficking defect | BrS, SCD, RBBB | 94 |
| S528A | Phosphorylation site | Decreased INaP: trafficking defect | BrS, SCD, RBBB | 94 |
| R811H | DII/S4 | Decreased INaP: negative shift of inactivation, slower recovery from inactivation | BrS | 82 |
| L812Q | DII/S4 | Decreased INaP, Iwindow: trafficking defect, negative shift of inactivation | BrS | 96 |
| I890T | P-loop of DII | Decreased INaP: positive shift of the activation | BrS | 80 |
| S910L | DII/S5-S6 | Decreased INaP: trafficking defect, positive shift of activation | BrS, DCM | 20 |
| R1023C | DII-DIII | No detected current | ERS, VF, structural myocardial alteration | 84 |
| A1055G | DI/S5-S6 | Decreased INaP: negative shift of inactivation, degradation | ERS | 100 |
| W1095X | | No detected current | BrS, epilepsy | 87 |
| S1218I | DIII/S1 | Complete loss of INaP: trafficking defect | BrS | 82 |
| D1275V | DIII/S3 | Decreased INaP: positive shift of activation, enhanced degradation, trafficking defect | CCD, DCM, SND, AT, VT | 105 |
| N1380del | DIII/S5-S6 | No detected current | CCD, VT | 103 |
| R1390H[a] | DIII/S4 | Decreased INaP: positive shift of activation, negative shift of inactivation, slower recovery from inactivation; increased INaL: slower deactivation | BrS, LQT, AA, VA | 99 |
| A1428S | DIII/S5-S6 | Decreased INaP | BrS | 97 |
| D1430N | DIII/S5-S6 | Complete loss of INaP: blockade of ion permeation | BrS, SCD | 86 |
| N1472del | DIII-DIV | Decreased INaP: positive shift of the activation and inactivation, slower recovery from inactivation | BrS, LQT, syncope, SCD, 2:1 AV block | 83 |
| 1493delK[a] | DIII-DIV | Decreased INaP: trafficking defect; enhanced recovery from inactivation | CCD, VA, SCD | 85 |
| R1629X | DIV/S4 | No detected current | BrS, in compound with p; A226V | 102 |
| R1632C | DIV/S4 | Decreased INaP: negative shift of inactivation, slower recovery from inactivation | BrS, sinus node dysfunction(SND) | 98 |
| D1690N | DIV/S5-S6 | Decreased INaP: positive shift of activation, slower recovery from inactivation; | BrS | 101 |
| G1748D | N-terminus of DIV/S6 | Decreased INaP: positive shift of activation curve, faster inactivation | BrS | 29 |
| F1775Lfs15[a] | DIV/S6 | Decreased INaP | overlap syndrome of SSS and BrS | 81 |
| L1786Q[a] | | Decreased INaP: positive shift of activation, negative shift of inactivation; increased INaL | overlap syndrome of LQT3 and BrS | 92 |
| D1790N | | No detected current | SSS | 89 |
| D1816VfsX7[a] | Truncation of C-terminus | Decreased INaP: trafficking defect, positive shift of activation; increased INaL: positive shift of inactivation, accelerated activation, faster recovery from inactivation | BrS, VF, bradycardia, AF | 90 |
| Q1832E | C-terminus | Decreased INaP: trafficking defect | BrS, SIDS, in compound with R1944△ | 104 |
| R1860Gfs12[a] | Truncation of C-terminus | Decreased INaP: negative shift of inactivation, degradation, positive shift of activation; increase INaL: delayed inactivation | SSS, AF, AVB | 93 |
| V2016M | SIV motif | Decreased INaP: trafficking defect, positive shift of activation | BrS | 95 |
| c; 4297G>C | DIII/S5-S6 | Decreased INaP: prolonged recovery from inactivation, positive shift of activation, affect translation process or degradation of the mutant protein | ERS | 88 |

[a]Both gain and loss of function mutations;
AA: atrial arrhythmia; AF: atrial fibrillation; AT: atrial tachycardia; AVB: atrioventricular block; BrS: Brugada syndrome; CCD: cardiac conduction disease; DCM: dilated cardiomyopathy; ERS: early repolarization syndrome; LQT: long QT syndrome; RBBB: right bundle branch block; SCD: sudden cardiac death; SIDS: sudden infant death syndrome; SND: sudden nocturnal death; SSS: sick sinus syndrome; SUNDS: sudden unexpected nocturnal death syndrome; TdP: Torsade de pointes; VA: ventricular arrhythmia; VT: ventricular tachycardia.

the AP morphology of epicardial versus endocardial cells.[11,110] Loss-of-function of SCN5A results in a reduction in $I_{Na\text{-}L}$; this alteration increases the relative amplitude of the fast, transient outward $K^+$ current ($I_{to}$), which is the most prominent in epicardial cells of the right ventricle.[111,112] Under normal conditions, $I_{to}$ inhibited the depolarizing effect of the $I_{Na\text{-}L}$ during the AP plateau, resulting in a marked AP notch in association with depolarizing $Ca^{2+}$ currents in a "spike-and-dome" morphology.[113] Consequently, the loss of $I_{Na\text{-}L}$ and increase in $I_{to}$ leads to a negative shift in the membrane potential, resulting in an "all-or-none" repolarization and causing an enhanced dispersion of repolarization in epicardial cells.[114] Finally, this results in premature repolarization, phase 2 reentry, and significant AP shortening. In contrast, endocardial cells show a much smaller $I_{to}$ and $I_{Na\text{-}L}$ reduction, which do not significantly affect AP morphology and duration. The transmural heterogeneity of the cellular membrane voltage ultimately causes ST-segment elevation, a J wave, and even severe "R on T," which leads to fatal VF on an ECG (Figure 3).[115] Previous studies showed that the alteration of channel kinetics, such as a faster speed of inactivation or a negative shift in the voltage-dependence of inactivation, causes a loss of function of $I_{Na\text{-}L}$ (Table 4).[116]

First, faster inactivation kinetics contribute to a decreased $I_{Na\text{-}L}$. For example, V1591L was detected in a BrS patient with a decreased $I_{Na\text{-}L}$ as a result of faster inactivation kinetics.[117] However, an R568H variant identified in a patient[42] diagnosed with QT prolongation also showed loss of function by faster inactivation kinetics. The mechanism of genotype-phenotype interactions remained unknown. Second, a negative shift of the voltage dependence of fast inactivation leading to a reduced $I_{Na\text{-}L}$ caused loss of function of SCN5A. T1620K[118,119] mutant channels in CCD patients, for instance, inactivate rapidly at less depolarized potentials, which may result in a significant reduction in $I_{Na\text{-}L}$ and consequently lead to delayed AP upstroke in the Purkinje system. Similarly, the R2012H variant in BrS patients also resulted in a loss of function due to a negative shift of the voltage-dependence of inactivation.[42]

## Loss-of-function mutations in both $I_{Na\text{-}P}$ and $I_{Na\text{-}L}$

Studies pointed out that most of the mutant Nav1.5 associated with a conduction disorder displayed either a drastic current reduction or shifts of steady-state inactivation/activation or both,[120,121] which may involve the alteration of $I_{Na\text{-}P}$ and $I_{Na\text{-}L}$ at the same time. As seen in G514C[122,123] mutant channels, a positive shift of both steady-state activation and inactivation, leading to changes in both $I_{Na\text{-}P}$ and $I_{Na\text{-}L}$, was observed and the shift of the activation curve predominated by only 3 mV, which was still sufficient to produce a reduced upstroke velocity. This type of mutation may cause overlap syndromes. It is reasonable to speculate that simultaneous alterations of inactivation/activation kinetics may lead to this type of phenotype.

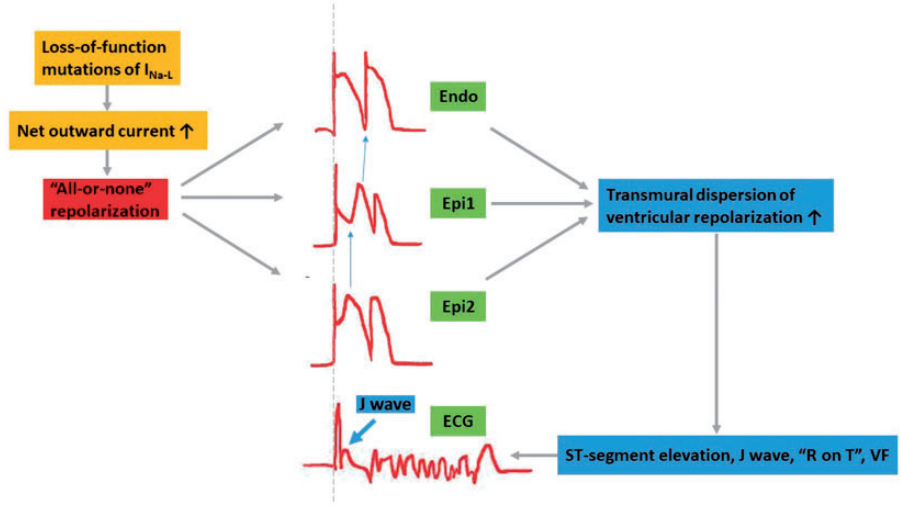


**Figure 3.** The ion mechanism of loss of function of $I_{Na\text{-}L}$ leading to arrhythmia.

**Table 4.** The newest identified loss of INa-L function of SCN5A mutations from 2013 to 2018 and their reported electrophysiological properties.

| Mutation | Protien domain | Biophysical properties of mutant protein | Cardiomyopathy and accompanied features | References |
|---|---|---|---|---|
| R1629Q | DIV/S4 | Decreased INaL: negative shift of inactivation, enhanced intermediate inactivation, prolonged recovery from inactivation | BrS, SCD | 116 |

BrS: Brugada syndrome; SCD: sudden cardiac death.

## Coexistence of gain- and loss-of function mutations

SCN5A mutations that present with an overlapped phenotype of LQT3 and BrS were also described.[124] In vitro studies suggested that these uncommon SCN5A mutations cause a mixed phenotype by altering the amplitude of $I_{Na\text{-}P}$ and $I_{Na\text{-}L}$ through enhanced sodium channel inactivation, a negative shift in steady-state sodium channel inactivation, and enhanced tonic block in response to sodium channel blockers.[78,125]

R1193Q,[126,127] E1784K,[128] and S216L variants in SCN5A are proposed to cause either BrS or LQTS. E1784K, originally described by Wei *et al.*[129] and subsequently explored by several other groups, is the most common mutation that causes both LQT3 and BrS phenotypes. $I_{Na\text{-}P}$ amplitude is significantly reduced, whereas the steady-state inactivation is shifted to much more positive potentials that lead to an enhanced $I_{Na\text{-}L}$. The R1193Q mutation reveals a slower inactivation with a persistent $I_{Na\text{-}L}$, which accounts for the gains of channel function and is in line with the LQT3 manifestations. Additionally, the steady-state inactivation of this mutation was shifted to a more negative voltage that lowered $I_{Na\text{-}L}$, which explains the loss of function of sodium current and BrS clinical characteristics. S216L is proposed to be an LQTS3-causing mutation because of a significant increase in the persistent $I_{Na\text{-}L}$, as well as an acceleration of the recovery from inactivation. It was also identified in a BrS patient due to a significant reduction in the $I_{Na\text{-}P}$. Therefore, S216L is considered to cause a mixed BrS/LQT phenotype. The mechanisms of the coexistence of gain and loss of channel functions remain unclear; studies speculate that some environmental factors may play a vital role in the formation of the phenotype, and there might be other gene mutations to identify.

## Perspectives

Since the first-generation gene sequencing technology was invented by Sanger in 1977, it has made tremendous progress. The brand-new third-generation sequencing technology has made the identification of gene mutations and genotypes more convenient. Functional analysis, including automatic patch clamp to study ion currents, in silico model simulation, and cryo-electronic microscopy to observe protein structure are advanced approaches to investigate the function of ion channels. New mutant models, such as iPSC-CM and transgenic animals, are more practical methods to investigate genotype–phenotype interactions. In clinical practice, we should also take more advantage of the current methods, such as ECG. ECG reflects the immediate manifestation of cardiac electrical activities on the body surface, provides the most direct evidence, and is a convenient approach for diagnosis. The alteration of T wave morphology and duration on the ECG is now used to distinguish different types of gene mutations. These discoveries stand out as notable landmarks in the progression of modern medical science that will allow further interpretations of the related pathogenesis. Additionally, in reference to these underlying mechanisms, many targeting therapies are desperately needed. Therapies that target these specific mutations with gene therapy, including RNA interference (RNAi) and CRISPER/Cas9, are under exploration, in addition to other traditional medicine therapies that affect the physiologic derangements of the mutations. The present findings enhance the general concept that the in vitro characterization of mutant ion channel functions is a key component for the generation of specific therapeutic strategies for patient management. Late sodium channel blockers, including mexiletine, ranolazine, flecainide, and a new compound, GS-6615, have been used in patients with LQT3 to restore the gain of channel function. Mexiletine also rescues the membrane expression of the Nav1.5 channel that expresses the BrS mutation. Mexiletine may be able to rescue the retention of Na1.5 in the ER to increase the sodium current, but it can also block the $I_{Na\text{-}L}$, which manifests as an inhibitory effect.

In this review, we summarized the gain and loss of channel functions in the SCN5A gene and discussed the underlying mechanisms of its genotype–phenotype relationship. The detailed mechanisms that underlie dysfunctional Nav1.5 due to SCN5A mutations are described herein, and we also provide some new evidence for additional approaches in the treatment of arrhythmias due to mutant Nav1.5 channels.

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